PSA in body fluids – an overview for users of the SERATEC PSA SEMIQUANT Tests

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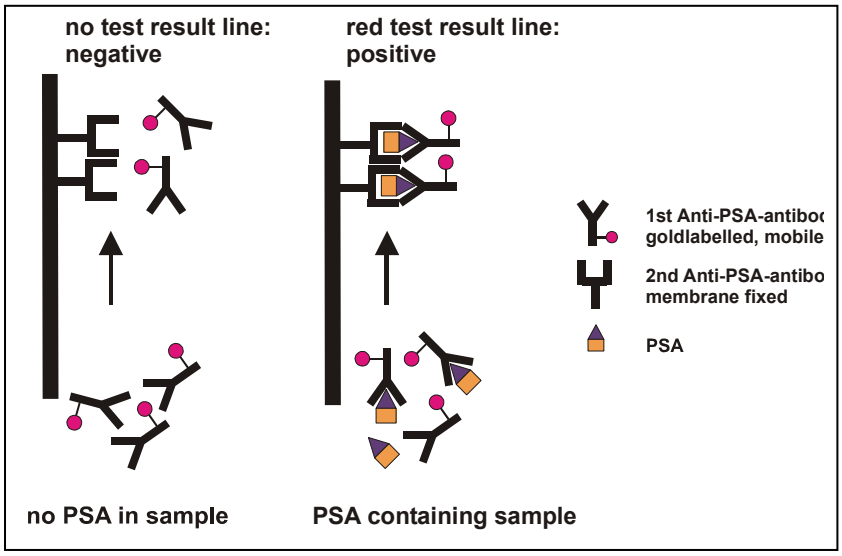
In recent times we frequently got inquiries from customers who wanted to know details about the presence of PSA in other body fluids than semen. Especially frequent were questions about weak positive results from vaginal or oral swabs.
To make an interpretation of test results easier we would like to provide the users of the SERATEC SEMIQUANT test with a summary of data found about PSA in the literature. We not only want to summarize the information about PSA concentrations in various body fluids but also would like to discuss matters of stability (if available). Further we would like to give recommendations concerning the sample preparation and the interpretation of results. If possible we will refer to the respective literature so that the reader can look up the original publications if interested.

1) Background

Seminal fluid contains high concentrations of PSA. The protein functions as a serine protease. It liquefies the seminal fluid by cleaving seminogelin thus allowing a sufficient motility of the sperms. Because of its extremely high concentration PSA has been used efficiently as a marker protein for seminal fluid in the forensic field. Unfortunately, the presence of PSA is not restricted to seminal fluid. PSA can also be detected in other male or female body fluids although the concentrations are generally dramatically lower than in seminal fluid. However, this fact poses the question how reliably positive (especially weak positive) PSA test results truly indicate the presence of semen.

2) Short Description of the PSA SEMIQUANT Test

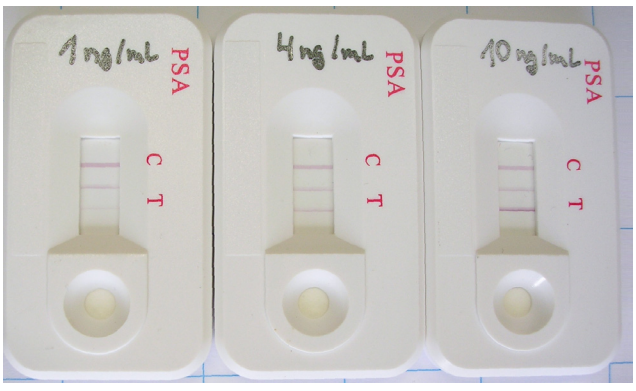
The PSA SEMIQUANT test is a sandwich immunoassay. PSA is detected by two monoclonal antibodies that form a sandwich complex with PSA, if it is present in the sample material. This complex can be seen on the membrane as the red test result line (T), allowing a visual interpretation of the test result. The following picture shows how the sandwich complex is formed.



The guaranteed detection limit of the test is 2 ng/ml PSA. However, generally the test is more sensitive. Usually PSA concentrations around 0.5 ng/ml still result in the formation of a weak test result line. An internal standard line with a defined color intensity (middle line) allows a semiquantitative interpretation of the test result. If the color intensity of the test result line matches that of the internal standard, the concentration of PSA in the sample material is around 4 ng/ml. More intense test result lines indicate higher concentrations, less intense test result lines indicate lower concentrations. The upper line (C) serves as an internal control for functionality and correct performance of the test. Like the internal standard line it should always appear because it is formed independent of the presence of PSA in the sample.

Brief Description of the SERATEC PSA SEMIQUANT Test

Intended Use:	Detection of Seminal Fluid by the Determination of PSA
Indication:	Sexual Assault Cases
Principle:	Chromatographic Sandwich Immunoassay
Range:	Lower Detection Limit: ~ 0,5 ng/ml (very weak test result line) Upper Detection Limit: ~ 100 µg/ml (no High Dose Hook Effect) at 500 µg/ml High Dose Hook Effect is clearly visible
Time:	10 minutes after addition of the sample the test result is interpreted visually
Performance Characteristics:	Diagnostic Sensitivity: 100% Diagnostic Specificity: 100%
Unit:	Box with 40 individually wrapped test devices including pipettes



Example for the semiquantitative interpretation of test results

- **General recommendations for the test procedure**

The test procedure is started by the addition of 200 µl of liquid into the round sample well. After an incubation time of 10 minutes at room temperature during which the red lines appear, the test result is interpreted visually.

Generally the sample material will be generated by the extraction of swabs or dried stains. Because the expected amount of seminal fluid, the place from which the sample has been taken, the age of the material etc. will vary considerably, it is difficult to give general recommendations concerning the preparation of sample material. We will try to discuss factors that might influence the preparation of the sample and the interpretation of results under the different topics.

Dependence on the pH
Studies in our laboratory showed, that the test result of the PSA SEMIQUANT test is influenced by the pH of the sample material. Low pH values (pH < 5) that are caused by **organic acids** (citric acid, acetic acid, oxalic acid) might lead to false positive test results. The lower the pH the more intense becomes the false positive test result line. Frequently the line is spotted and is not formed uniformly across the whole width of the test membrane. Interestingly, this phenomenon is **only** observed **at low pH in the presence of organic acids**. If the pH of other buffer solutions (see table below) is adjusted with HCl, no false positive results are observed (up to pH 3). Between pH 5 to pH 10 no false positive results occur in any case. In this range the sensitivity of the test remains constant. The color intensity of the test result line is equal to that of the internal standard line at a PSA concentration of 4 ng/ml. Only with 50 mM Tris pH 10 the test result line appeared slightly weaker than the internal standard. With Tris pH 9 the test result with 4 ng/ml PSA was as expected.

In any case we recommend to prepare the sample material in a way, that the pH is neutral or close to neutral. If possible buffer solutions instead of distilled water should be used for the extraction to avoid changes in the pH due to the sample material. In some cases it might be necessary to check the pH of the liquid with a small piece of pH indicator paper. In the future we will test more organic acids and/or different immunoassays to see if the false positive results with organic acids at low pH are a general phenomenon.

pH- dependence of the PSA SEMIQUANT test

pH[1]	10 mM HEPES		50 mM Tris		PBS		50 mM citric acid		50 mM oxalic acid		50 mM acetic acid	
	without PSA	4 ng/ml PSA	without PSA	4 ng/ml PSA	without PSA	4 ng/ml PSA	without PSA	4 ng/ml PSA	without PSA	4 ng/ml PSA	without PSA	4 ng/ml PSA
3	-	IS=T	-	IS=T	-	IS=T	+[2]	n.d.	+[2]	n.d.	+[2]	n.d.
4	-	IS=T	-	IS=T	-	IS=T	+[2]	n.d.	+[2]	n.d.	+[2]	n.d.
5	-	IS=T	-	IS=T	-	IS=T	-	IS=T	-	IS=T	-	IS=T
6	-	IS=T	-	IS=T	-	IS=T	-	IS=T	-	IS=T	-	IS=T
7	-	IS=T	-	IS=T	-	IS=T	-	IS=T	-	IS=T	-	IS=T
8	-	IS=T	-	IS=T	-	IS=T	-	IS=T	-	IS=T	-	IS=T
9	-	IS=T	-	IS=T	-	IS=T	-	IS=T	-	IS=T	-	IS=T
10	-	IS=T	-	IS>T	-	IS=T	-	IS=T	-	IS=T	-	IS=T

1: the pH value of the solutions was adjusted with NaOH or HCl
2: the test result line of the false positive results frequently looked spotty and was not uniformly formed across the whole width of the membrane.
IS = Internal Standard T = Test Result Line n.d. = not determined

3) Presence of PSA in various body fluids / recommendations for the test procedure

Initially PSA was thought to be a highly prostate specific protein. However, in the last years PSA was detected in a variety of male and female tissues and body fluids. In this summary we provide a list of the PSA concentrations found in different body fluids. We also would like to discuss if these "background concentrations" might influence the result of the PSA SEMIQUANT test. Seminal fluid contains by far the highest PSA concentration compared to any other body fluid. However, in forensic case material it is not known how much (if at all) seminal fluid is to be expected and how much of the PSA has been lost due to drainage or degradation.

Therefore it is important to know as much about the sexual assault case as possible e.g.:

- How much time has passed between the assault and the withdrawal of the sample?
- Hygienic measures taken by the victim (e.g. showering, bathing etc.)
- Did an ejaculation take place? If yes, where (vaginal, oral, rectal, outside body orifice)
- Did the assailant use a condom?
- Last date of consensual coitus ...etc.

The combination of this knowledge together with the knowledge of naturally occurring PSA concentration in body fluids and data about the stability of PSA should make it easier to interpret positive PSA test results. We hope that you will find this detailed summary helpful to judge if seminal fluid is present or not.

3 1) Seminal Fluid

- **PSA concentration in seminal fluid according to the Literature**

Generally seminal fluid is secreted during an ejaculation. For healthy fertile men the amount is between 2 – 6 ml that contain approximately 40 million sperms per ml. In oligospermic individuals the amount of sperm cells is dramatically lower. In the semen of aspermic or vasectomized individuals no sperm cells are detectable. Also in the liquid serving the lubrification that is secreted before the ejaculation sperm cells are hardly detectable. Independent of the amount of sperm cells all these fluids contain high amounts of PSA. According to the literature the concentration of PSA in seminal fluid is between 0.2 and 5.5 mg/ml (summarized value from Hochmeister et al., 1999). Lövgren et al. (1999) found an average value of 0.82 ± 0,22 mg PSA/ml (range 0,07-2,16 mg/ml) for PSA in seminal fluid.

- **Stability of PSA**

In the dried state PSA is a very stable protein. From dried semen stains that had been stored at room temperature for a long time PSA could be extracted at concentrations that were detectable with the PSA SEMIQUANT test. The oldest stain from which PSA was successfully recovered was 30 years old (Hochmeister et al., 1999).

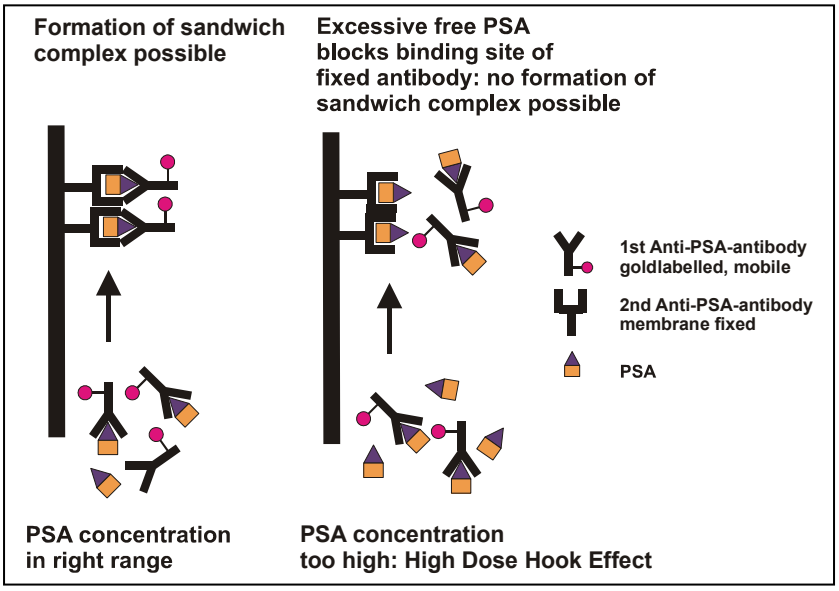
In the body of the victim PSA will be constantly diluted by other body fluids and will be lost due to drainage or degradation. We will discuss the stability of PSA in the vagina later under point "3.2 Vaginal fluid".

- **Results obtained with the PSA SEMIQUANT test**

As expected the PSA SEMIQUANT test shows positive test results with seminal fluid. Dilutions up to 2×10^{-6} (Sato et al., 2002) or $1,3 \times 10^{-6}$ (Hochmeister et al, 1999), respectively, could be detected with the test. If seminal fluid is not sufficiently diluted, there is the danger of generating false negative results due to a high dose hook effect (also called prozone effect). Hochmeister et al. (1999

recommend to dilute seminal fluid at least 1:100 to avoid a high dose hook effect. The following picture shows how a high dose hook effect is generated:

High dose hook effect
If there is an excess amount of PSA in the sample, PSA will not be bound completely to the gold-labeled antibody. Free PSA will reach the test result zone and bind to the PSA antibody fixed in this zone. The binding sites of the antibody become blocked so that the PSA bound to the gold-labeled antibody can no longer bind. The formation of the sandwich complex is repressed and no red test result line is formed.



Extraction of old stains
Various old stains (2-30 years old) from case material were extracted and determined with the PSA SEMIQUANT test. All showed positive test results (Hochmeister et al., 1999). Sato et al. (2002) tested a 4 year old semen stain. Also here the test result was positive.

- **Specificity of the PSA SEMIQUANT test**

Seminal fluid of the following mammals were tested **negative** by Hochmeister et al (1999) with the PSA SEMIQUANT test up to serial dilutions of 10^{-7} : cat, dog, pig, bull, and horse. Sato et al (2002) confirmed the negative test results with semen from cat, dog, pig, and bull. A cross reactivity with the above mentioned species can therefore be excluded.

- **Recommendations for the test procedure**

Generally the following can be said: If it is expected that the sample material contains a huge amount of seminal fluid but the test result is negative, a high dose hook effect should be ruled out by testing higher dilutions. We recommend buffer solutions at a neutral pH like HEPES, TRIS, TBS or PBS for diluting the sample.

Extraction
Generally, a small amount of material will be cut out of the middle of the stain for extraction . The size of the cuttings will be between 0.25 cm^2 (0.5 cm x 0.5 cm) and 1 cm^2 (1 cm x 1 cm) as a maximum. Hochmeister et al. (1999) extracted the cuttings with HEPES buffered saline for 2 hours at 4 °C on a shaker. Afterwards the sample was centrifuged and 200 µl the supernatant were used for the test. Gartside et al. (1993) describe that the extraction efficiency in HEPES buffer is slightly elevated if compared to water. Laux et al. (2003) also used HEPES buffer (0.24 %, pH 7.2) for the extraction.
We recommend to extract sample material in a neutral buffer instead of water if possible. Next to the slightly improved extraction efficiency there is the advantage that changes in the pH value due to the sample material will be minimized. The temperature at which the extraction takes place (4 °C or room temperature) does not seem to be critical. Please ensure, that the extracted material has not come into direct contact with other forensic detection aids e.g. the acid phosphatase (ACP) test.

Difficulties in estimating the amount of seminal fluid from extracted stains

For the extraction of stains the case material will be very inhomogeneous (different fabrics, different age/size of the stain). Therefore it is difficult to estimate the amount of PSA that is expected if the stain contains semen. Laux and Custis (2004) performed a nice experiment that makes it easier to estimate PSA amounts in stains caused by liquids. They tried to correlate the size of the stain with the applied volume of the liquid on cotton fabric and Whatman paper. They found out that a stain of 1 cm^2 is approximately caused by a volume of 10 µl of liquid. If this stain is cut out completely and is extracted in 1 ml of buffer the dilution factor of the sample would be 1:100. If the concentration of PSA is between 0.2 and 5.5 mg/ml in semen the calculated concentration of the sample would be between 2-55 µg PSA/ml if the extraction efficiency is 100%. Within this range no high dose hook effect is to be expected with the PSA SEMIQUANT assay.
Futhermore Gartside et al. (2003) found that the extraction efficiency for semen stains on cotton fabric is drastically lower than 100 %. The stains were extracted with an estimated efficiency of 0,34 % in water and 1,03 % in HEPES buffer, respectively. Gartside et al. performed the experiment in the following way: 500 µl of a sample that contained approximately 1.8 mg/ml PSA were given on cotton fabric causing a stain with a size of around 1555 mm^2. Out of the middle of the stain a 25 mm^2 piece was cut and extracted for 2 hours at room temperature in a volume of 250 µl. Calculating the expected amount at an extraction efficiency of 100 %, the supernatant should contain around 4 ng PSA /ml if diluted 1:14,500. However, in the experiment the test result line of the PSA SEMIQUANT test only showed a color intensity that exceeded that of the internal standard up to a dilution of 1:100.
In contrast, Hochmeister et al (1997) described that some supernatants of extracted stains resulted in false negative results due to a high dose hook effect. Only after diluting the supernatant 1:100 the test result became positive.
The above described examples show how difficult it is to estimate the amount and to give general recommendations for the sample preparation with semen stains. The supernatants of most stains should not lead to any high dose hook effects. However, clear results can sometimes only be obtained if additional dilutions of a supernatant are tested.

3.2) Vaginal fluid

Next to seminal fluid vaginal swabs will of course contain vaginal fluid. Therefore we would like to summarize the data that describe the naturally occurring PSA concentrations in the vaginal fluid. The possibility of wrong positive test results caused by background concentrations of PSA in vaginal fluid will be discussed taking into consideration the way of the sample preparation.

- **Concentration of PSA in the vaginal fluid according to the literature**

PSA is detectable in vaginal fluid only at low concentrations. The values for PSA concentrations in the vaginal fluid have been taken from studies determining if PSA could be used as a marker to test the failure rate of condoms. In these studies small amounts of semen were applied into the vagina of abstinent (at least for 48 hours) female volunteers. Afterwards changes in the PSA content of the vaginal fluid were monitored. In a study of Lawson et al. (1998) 20 couples were determined. Before the addition of semen the PSA-content of the vaginal fluid was low (median 0.11 ng/ml; range 0,00-1.25 ng/ml). Similar values were found by Macaluso et al. (1999). The vaginal fluid of 40 women, 27 of which were taking the pill, was determined at three different time points, before different amounts of their partners semen was applied into the vagina. The determination of the PSA concentration of the vaginal secret was done in duplicate and showed the following values: 0.43 (95% CI 0.26-0.71), 0.45 (95% CI 0.27-0.74) und 0.88 (95% CI 0.52-1.50). 48 hours after the application of semen the background concentrations were reached again: 0.28 (95% CI 0.17-0.46), 0.39 (95% CI 0.23-0.64) und

0.7 (95% CI 0.4-1.30) independent on the amount of semen applied (10 µl –1000 µl). Unfortunately the maximum values out of the 95% Confidential Interval could not be found in the publication.
Nevertheless these values show, that the PSA concentration is 10^{-4} to 10^{-6} fold lower in vaginal fluid than in seminal fluid. Furthermore the experiment showed that the application of only 10 µl of semen resulted in an approximately 200fold elevation of the PSA concentration in vaginal fluid.

- **Stability of PSA in the vagina**

Studies by Macaluso et al. (1999) show how the amount of PSA in the vaginal fluid decreases over the time, after women have been inseminated with different amounts of their partner's semen. The inoculation volumes were 10, 100 and 1000 µl, respectively. For the 10 and the 100 µl the PSA concentration in the vagina declined to background levels (3-9 % of samples > 1 ng PSA/ml) within 24 hours. At this time point the 1000 µl inoculum still resulted in 29 % of samples > 1 ng/ml and 8 % of samples > 5 ng/ml. Background levels were here reached at 48 hours.
Hochmeister et al. (1997) report, that PSA is detectable in the vagina for around 14-47 hours after a sexual assault. The stability of PSA is generally higher than that of the Acid Phosphatase (ACP). Sperm cells are detectable for the longest period (maximal several days). Kamenev et al. (1989) detected postcoital PSA for 10.5 –24 hours. Detection times by Graves et al. (1985) were given with 13 hours (minimum) to 47 (maximum) hours (average value 27 hours).

- **Results with the PSA SEMIQUANT test**

Vaginal swabs of abstinent women
Several authors determined vaginal swabs of abstinent women with the PSA SEMIQUANT test of SERATEC. In almost all cases the test results were negative. One exception was described by Denison et al. (2004), who found one positive result when testing 10 different female donors. Interestingly, the positive test results correlated with the menstrual cycle of the donor. Positive results were exclusively obtained three days before or during the menstruation (results were taken over three cycles). This result caused Kafarowski et al. (2004) to expand the study. Vaginal swabs of 70 female volunteers were tested. Samples were taken throughout the whole menstrual cycle. 130 vaginal swabs showed negative test results. No exceptions were found. 23 of the swabs were ACP positive, which shows that PSA is a more specific marker than acid phosphatase. Sperm cells were detected in none of the samples. Hochmeister et al. (1999) tested 10 vaginal swabs that has been diluted up to 10^{-4} all with negative results.

Case material
Hochmeister et al (1999) testet vaginal swabs of sexual assault victims (n=50). 42 of these swabs showed positive results whereas 8 tested negative with the PSA test. An ACP test that was performed in parallel showed 35 positive and 15 negative results. The discrepancy might be due to the different stabilities of PSA and ACP.

- **Recommendations for the test procedure**

Extraction
For the extraction of vaginal swabs we also recommend to use buffered solutions to keep the pH in a neutral range. Generally swabs or part of the swabs should be extracted in the same way as stains (shake 2 h at 4°C or room temperature, centrifuge briefly, use 200 µl of supernatant for testing).

Interpretation of test results: Is it likely to get positive test results due to background concentrations of PSA in vaginal fluid with the PSA SEMIQUANT Assay?
Laux and Custis (2004) published an interesting study in the internet dealing with PSA-concentrations in various body fluids and their possible influence on the result of PSA tests. The authors put a special emphasis on the way how sample material is gained, how it is extracted and what kind o dilution factor

of the original material is involved until the final measurement with the PSA test takes place. For swabs the authors found that the brand used in their lab could hold a volume of 125-150 µl until saturation. Afterwards the swabs were extracted in 1000 µl of HEPES buffer so that the minimal dilution factor was 0.15. If the sensitivity of the PSA SEMIQUANT is 0.5 ng PSA/ml and the dilution factor is 0.15, vaginal fluid must contain 3.3 ng PSA/ml to see a very faint test result line with the PSA SEMIQUANT test. This value is only true, if the efficiency of the extraction is 100%. In fact the extraction efficiency for air dried swabs seems to much lower. Laux and Custis (2004) described an extraction efficiency of 16%, whereas Gartside et al. (2003) only recovered 0,11 % .
One have to keep in mind that the PSA concentration given in the literature are no absolute concentrations because the data by Lawson et al. (1998) and Macaluso et al. (1999) are also based on extracted swabs. Here the air dried swabs (capacity of liquid uptake 1 ml) were extracted for 15 minutes in 3 ml of saline. The swab was pressed to the wall of the tube and removed. The remaining supernatant (approximately 2 ml) was used for the PSA measurement. The published PSA values represent the concentration of this supernatant. Corrections for the estimated dilution factor or an extraction efficiency have not been taken into account.
Graves et al. (1985) determined the PSA concentration of the vaginal fluid before the coitus in a different way. The weight of tampons was determined before and after removal from the vagina. The tampons were extracted in distilled water, centrifuged and the supernatant lyophilized. Afterwards the pellet was resuspended in the volume of liquid corresponding to the weight difference. Here the correction for a dilution factor is not necessary. However the reconstituted volumes were so small that the samples had to be diluted for the assay. Taking the detection limit of the assay into consideration it could only be concluded that the vaginal fluid contained less than 10 ng/ml PSA.
If 10 ng PSA/ml were taken is a hypothetical upper limit for vaginal fluid and the dilution factor would be 0.15 the PSA concentration in the sample would be around 1.5 ng/ml as a maximum. This would result in a weak test result line. Unfortunately, differences in the extractions efficiencies due to different methods can not be considered in these calculation examples.
The unexpected positive test result of Denison et al. (2004) might be partly explained by the fact that the vaginal swab was extracted in only 350 µl of H_2O. If the capacity of liquid uptake for the swab was similar as described by Laux and Custis (2004), the estimated dilution factor would be 0.43. If one assume 10 ng PSA/ml as upper limit for vaginal fluid this would correspond to a PSA concentration of 4.3 ng/ml in the sample. Unfortunately, we do not know the intensity of the test result line seen by Denison et al. (2004). Interestingly the raise in PSA concentration shortly before and during the menstruation in the vaginal fluid in the volunteer does not correspond with the time course of PSA levels over the menstrual cycle described by Aksoy et al. (2002). Here the highest values for PSA in serum and saliva were found in the follicular phase and the midcycle (see below).
Nevertheless the extension of the study by Kafarowski et al. (2004) with a lot of more volunteers exhibiting all negative test results, indicates that such extreme high PSA-levels in the vaginal fluid seem to be very rare.

If you use the PSA SEMIQUANT test routinely in your lab, we recommend to calculate the dilution factor during the sample preparation as described above. The capacity of the swab can be measured by adding water to the tip until it is saturated an can hold no more water. By dividing this value with the volume used for the extraction you can calculate the dilution factor of the case material (Laux and Custis, 2004). By this it becomes easier to compare your result to the values found about PSA in vaginal fluid in the literature.

Strong positive test results that drastically exceed the 4 ng/ml internal standard line are a strong evidence for the presence of semen. If the test result line is only weak, it can not be excluded, that it is caused by PSA in the vaginal fluid. However, as such high background concentration of PSA in the vaginal fluid seem to be very rare, it can still be seen as a pretty strong indicator that semen might be present.

3.3) Saliva

Next to seminal fluid oral swabs will of course contain saliva. Therefore we would like to summarize what is known about naturally occurring PSA concentrations in saliva. The possibility of "wrong positive" test results caused by background concentrations of PSA in saliva will be discussed taking into consideration the way of the sample preparation.

- **Concentrations of PSA in saliva according to the literature**

The PSA concentrations in saliva are very low. The described values range from < 0.03 ng/ml (Lövgren et al., 1999; 10 women, 6 men) to 0.02-0.34 ng/ml (Manello et al., 1996). Manello et al. determined a group of 40 female volunteers half of which were taking contraceptives (0.5 mg Gestoden, 0.035 mg Ethinylestradiol per pill). Interestingly, the group taking the contraceptive exhibited the highest concentration of PSA in saliva (average 0.099 ± 0.016, range 0.04- 0.34). In the control group the average value was 0.048 ± 0.007 (range 0.02-0.15). The PSA concentrations in serum did not differ between the two groups. Aksoy er al. (2002) determined changes in the PSA concentration in serum and saliva over the menstrual cycle. The highest values were found on day 9 (follicular phase) and day 14 (midcycle) with values of 0.024 ± 0.011 and 0.029 ± 0.013 ng PSA /ml, respectively. No PSA concentrations larger than 0.06 ng/ml were found on this study.
A pretty old abstract of Breul et al (1993) does not fit into the picture of low PSA concentrations in saliva. Here extremely high concentrations of PSA were found in the saliva of 165 patients (20 women, 39 patients with benign prostate hyperplasia, 24 patients with localized prostate carcinoma, 17 with metastasizing prostate carcinoma, 14 patients after transurethral manipulation, and 51 patients with other urological diseases) with the values ranging between 129 – 688 ng PSA/ml. These values did not correlate with the serum concentrations. We do not know what caused these high values. So far we got no answers to our inquiries. Maybe the assay (Hybritech Assay) showed a cross reactivity with another protein in the saliva? Taking into account our own experiences with saliva samples and the PSA SEMIQUANT test and the high number of correspondingly low PSA concentrations reported by different authors, we feel that the high values published by Breul et al. (1993) are somehow doubtful.

- **Stability of PSA**

We could not find any data about the stability of PSA in the oral cavity. Therefore we do not know how long PSA is detectable in the oral cavity after oral intercourse. For sperm cells we found the following guidelines (taken from Medico-Legal Aspects of Sexual Offences, page 61): up to 9 hours (lips of mouth) and up to 6 hours for oral swabs. If one assume that the loss of PSA is due to dilution and drainage rather than degradation similar times might be true for PSA.

- **Results with the PSA SEMIQUANT test**

Different labs determined saliva samples with the PSA SEMIQUANT test. In all cases negative test results were obtained. Hochmeister et al. (1999) tested saliva samples of 10 male and 10 female volunteers all with negative results. Male saliva samples were also determined as negative by Sato et al. (2002). Denison et al. (2004) tested a variety of biological materials including saliva with the test and got negative results.

- **Recommendations for the test procedure and the interpretation of test results**

Extractions of oral swabs should be carried out in the same way as described for vaginal swabs. The dilution factor for the saliva should therefore also be 0.15. Because the naturally occurring PSA concentrations are very low (highest value 0.34 ng PSA/ml) there is no danger of positive test results caused by saliva even if the sample material would no be diluted. Nevertheless saliva should not be used directly in the assay because of its high viscosity. Again we recommend to use a buffer with neutral pH for the extraction. In some cases it might be useful to check the pH with a small piece of indicator paper especially if the victim has thrown up during/after the sexual assault.

3.4) Urine

In sexual assault cases it is not unlikely that the case material that should be examined for the presence of seminal fluid also contains urine of the victim. Here we want to summarize the data about PSA concentrations in urine that we found in the literature. We will also discuss the possibility of "wrong positive" test results caused by background concentrations of PSA in urine taking into consideration the way of the sample preparation.

- **Concentrations of PSA in urine according to the literature**

Female urine
A detailed study about the presence of PSA in female urine samples was carried out by Schmidt et al. (2001). Urine samples of 217 women were determined. PSA was detectable in only 11 % of the samples These samples contained an average amount of 0.29 ng PSA /ml ranging between 0.12-1.06 ng/ml. Breul et al. (1997) describe higher PSA values for female urine samples. The specimen contained an average of 1.73 ± 1.68 for the control group. The other group of patients who wanted to undergo a gender shift and took high doses of testosterone exhibited PSA values of 12.03 ± 10.47 ng/ml in the urine. Interestingly, the serum concentrations of PSA were the same in both groups (0.12 ± 0.02 and 0.16 ± 0.07 ng/ml, respectively). In the abstract from 1993 that described the high PSA concentrations for saliva (see above), Breul et al. report an average value of 7.36 for female urine (n=20). Another average value from Breul et al. (1994) is given with 3.72 ng/ml. Manello et al. (1998) report elevated PSA levels in urine of women taking oral contraceptives (Milvane, Schering). Here 92% of the samples showed PSA levels within the detectable range (median 0.451 ng/ml, average 0.521 ± 0.05 ng/ml, range 0.09-1.239 ng/ml). In contrast the control group showed 80% of samples within the detection range of the test with consistently lower values (median 0,035 ng/ml, average 0.038 ± 0.005 ng/ml, range 0.02-0.15 ng/ml). The women had lived sexually abstinent for at least one week prior to the sample taking. Interestingly, there was no correlation found for the PSA concentrations in serum. Another publication determined the PSA concentration in urine from healthy women versus women with Polycystic Ovary Syndrome (PCOS). Whereas the control group (n=41) exhibited very low values (average: 0.0043 ± 0.0018 ng/ml, range 0-0.046 ng/ml), the PCOS group (n=37) showed strongly elevated urine levels of PSA (average: 0.820 ± 0.344 ng/ml, range <1-10.289 ng/ml). The PSA found in the urine is probably synthesized locally in the periurethral glands (skene's gland). Also other tissues of the urogenital tract may contribute to the PSA level of female urine.

Male urine
Sato et al. (2002) estimated that the maximal concentration of PSA in male urine is around 800 ng/ml using the semiquantitative PSA SEMIQUANT test. Urine of boys 12 years or older may show positive test results. This observation is in line with the result of Antoniou et al. (2004) who found that the serum PSA level of boys becomes elevated at an age of 12. At this age the prostate begins to develop.
Iwakiri et al. (1993) determined the urine of 18 patients suffering from an adenocarcinoma of the prostate. Some of these patients showed extremely high PSA values in the urine (average 915.1 ng/ml, range 21 –2,853 ng/ml), the first voided urine generally exhibiting higher concentrations than the midstream urine. Interestingly, the values remained pretty high (mean 21,4 ng/ml) even after removal of the prostate indicating that at least a part of the PSA in male urine is synthesized locally.

- **Results with the PSA SEMIQUANT assay**

Hochmeister et al. (1999) determined 10 urine samples of female volunteers with the PSA SEMIQUANT test. All showed negative test results up to an dilution of 10^{-3}. Of 10 male urine samples some exhibited positive and some negative test results as expected. Sato et al. (2002) obtained consistently positive test results with undiluted male urine samples (n=17, age > 21 years). The highest dilution at which one of the male urine samples still tested positive was 1:200. Urine samples of boys aged 12 or older may contain PSA levels that are detectable with the PSA SEMIQUANT test (Sato et al., 2002). Dried urine stains on filter paper (1 cm^2 pieces extracted with 500 µl T-TBS) showed positive test results after storage for 4 weeks at room temperature. Denison et al. (2004) tested female urine samples with negative results.

- **Recommendations for the Test Procedure and Interpretation of Results**

It cannot be excluded that undiluted female urine may cause positive test results with the PSA SEMIQUANT Assay. However, generally the sample material will be obtained by the extraction of stains. If stains are extracted the estimated dilution factor is high enough (1:100, if a 1 cm^2 stain caused by 10 µl of liquid is extracted in 1 ml of buffer; see extraction of stains under "Seminal fluid"), so that false positive results due to female urine in the sample material can almost certainly be ruled out.

In contrast male urine samples may lead to positive test results even if they are diluted. Taking into consideration a dilution of 1:100 during the extraction (see above) it is still possible to get a positive result with the PSA SEMIQUANT assay. If low extraction efficiencies are assumed as described by Gartside et al. (2003) for semen stains, positive test results with extracted stains due to male urine seem to be unlikely. However, Sato et al. (2002) could extract PSA from male urine stains on Whatman paper even though the dilution during the extraction was approximately 1:50. This might mean that the extraction from Whatman paper is more successful than from cotton fabric and/or that the results from Gartside et al. cannot be generalized because the extraction methods differed too much (different extraction volume, different buffer, semen instead of urine).
If it is necessary to discriminate between male urine and semen, you might try to test higher dilutions at which clear conclusions are possible. Alternatively, additional tests can be used (see Hochmeister et al., 1997).

3.5) Serum

Case material from sexual assaults may contain blood of the victim. In order to check if the naturally occurring PSA content of blood may influence the result of the PSA SEMIQUANT assay this paragraph will summarize the data about the PSA content in female serum. We also would like to discuss briefly the amount of PSA found in male serum. The possibility of "wrong positive" test results caused by background concentrations of PSA in serum will be discussed taking into consideration the way of the sample preparation

- **PSA Concentrations in serum according to the literature**

Female Serum
Generally the PSA concentration in female blood is very low. Manello et al. (1996) determined the PSA content in the serum of 40 women half of which were taking the pill. The average PSA-value found was 0.046 ± 0.07 ng/ml (range: 0.02 – 0.16 ng/ml). The taking of oral contraceptives had no influence on the PSA concentration in serum. Filella et al. (1996) measured the PSA concentration in 276 female sera. In 58% of the samples no PSA could be detected. Only 3 of the samples showed values > 0.1 ng/ml but remained below 0.6 ng/ml. In another study by Aksoy et al. (2002) the PSA

concentration was determined during the menstrual cycle. The highest values were measured in the follicular phase and the midcycle with values of 0.032 ± 0.014 and 0,035 ± 0.015 ng/ml, respectively. During a gender shift study Breul et al. (1997) found average serum concentration of 0.12 ± 0.02 ng PSA /ml for the control group and 0.17 ± 0.07 ng/ml for the group taking testosterone. Melegos et al. (2005) describe PSA as a serum marker for elevated androgen levels in women suffering from hirsutismus. The highest concentration described was 0.579 ng PSA /ml. The values of the control group were clearly below this with values between 0 – 0.019 ng PSA / ml.

Serum of Children
Antoniou et al. (2005) dertermined the PSA concentration in the serum of children. Up to an age of 144 months girls and boys showed consistently low PSA concentrations of maximally 0.0064 ng/ml. Whereas the values of the girls >144 months remained consistently low, the boys showed a clear elevation of PSA in the serum (0.0141-0.143 ng/ml). The developing prostate was supposed to be the reason for the higher PSA values.

Male Serum
Healthy men show PSA levles of < 4 ng/ml in the their serum. In a lot of male blood samples the PSA content is below the detection limit of the PSA SEMIQUANT assay (SERATEC, own results). Generally it can be said that older men have higher PSA levels than younger men. The PSA concentrations may reach values of > 200 ng/ml if a patient suffers from prostate carcinoma.

- **Results with the PSA SEMIQUANT Assay**

The vaginal swabs taken by Hochmeister et al. (1999) were partly "contaminated" with menstrual blood. They all showed negative test results with the PSA SEMIQUANT assay. Sato et al. (2002) tested blood samples of male volunteers with negative results. Blood samples of 3 women determined by Laux and Custis (2004) showed negative results as expected. Also the male and female blood samples tested by Denison et al. (2004) consistently showed negative test results.

- **Recommendations for the Test Procedure and the Interpretation of Results**

In very rare cases undiluted female serum may cause positive results with the PSA SEMIQUANT assay. For the forensic use this should cause no problem as here stains or swabs will be extracted. During the extaction the case material is sufficiently diluted the estimated dilution factor for stains being 1:100 (1 cm^2 stain extracted in 1 ml of buffer) and for swabs being 0.15. Therefore it can almost certainly be ruled out that female blood interferes with the test result.

The same is true for the blood of healthy men. However, progressed diseases of the prostate may lead to PSA concentration in the serum that might be detectable with the PSA SEMIQUANT assay even if diluted 1:100. If in doubt, it might be necessary to test higher dilutions.

3.6) Other biological materials

Also other biological materials has been determined for their PSA content or have been tested with the PSA SEMIQUANT assay. The following table briefly summarizes the available data.

Presence of PSA in other biological materials

Material	**Concentration of PSA ng/ml**	**Result of with PSA SEMIQUANT**	**source**
Amniotic fluid	maximal value: 8.98 ng/ml (Peak in 3rd trimester, after that slowly decreasing)		Lövgren et al. (1999)
Amniotic fluid (18 samples)	Maximal value: 0.9 ng/ml		Filella et al. (1996)
Breast milk [1]	Maximal value: 2100 ng/ml average: around 1 ng/ml (highest value directly after birth for ca, 4 days, after this decrease of non-detectable to slightly elevated		Lövgren et al. (1999)
Breast milk [1] (16 lactating women)	Maximal value: ~ 110 ng/ml; non-detectable in 12.5 % of the samples		Filella et al. (1996)
Breast secretions (6 non-lactating women)	Maximal value: ~ 30 ng/ml Remaining samples between < 3 und > 0.02		Filella et al. (1996)
Breast milk and urine samples of 5 lactating mothers		All negative test results	Laux et Custis (2004)
Fecal material (swabs), 10 female and 10 male		All negative test results	Hochmeister et al. (1999)
Anal/Rectal swabs		All negative test results	Denison et al. (2004)
perspiration (swabs), 10 female and 10 male		All negative test results	Hochmeister et al. (1999)
Microorganisms[2]			
Bacillus *Clostridium* *Pseudomonas* *E. coli* *Staphylococcus* *Candida albicans*		All negative test results	Hochmeister et al. (1999)

1: Bosco and Hapack (2001) detected PSA in the diaper of a nursed child and assumed that it originated from colostrum in breast milk

2: overnight cultures were centrifuged and the pellet resuspended in 1 ml of HEPES buffered saline

4) Summary

The following table summarizes the PSA concentrations of the most important female body fluids that might be part of forensic case material. Only the **maximum values** of healthy women that we found in the literature are given in the table. Please keep in mind that some diseases or hormonal treatments like gender shift (elevated testosterone levels), hirsutism with elevated androgen levels or PCOS might influence the PSA content of some body fluids.

Summary: PSA concentrations in the forensically most important female body fluids

Body fluid (female)	Maximum value found in literature ng/ml[a]	Preparation of Samples Dilution factor for swabs: 0,15[h] Dilution factor for stains: 0,01[i]	Expected test result with the PSA SEMIQUANT Assay
Saliva	0.34 [b]	Extraction of swabs Extraction of stains	Negative Negative
Blood (serum)	< 0.6 [c]	Extraction of swabs Extraction of stains	Negative Negative
Urine	1.239 [d] (using oral contraceptives)	undiluted Extraction of swabs Extraction of stains	Positive results possible[j] Negative Negative
	7.36 (average)[e]	undiluted Extraction of swabs Extraction of stains	Positive results possible[j] Weak positive results possible Negative
Vaginal fluid	1.5 [f]	Extraction of swabs	Weak positive results possible [k]
	< 10 [g]	Extraction of stains Extraction of swabs Extraction of stains	Negative Weak positive results possible [k] Negative

a: respective maximum values, if an average value is given it will be listed in the table
b: Manello et al., 1996 (values from Breul et al. 1993 are not considered)
c: Fillela et al., 1996
d: Manello et al., 1998
e: this high PSA concentration from Breul et al. (1993) is not in line with the PSA concentrations found in the majority of the studies
f: Macaluso et al., 1999, extracted supernatant from vaginal swabs, estimated dilution factor 1:3
g: Graves et al., 1985 (Detection limit of the assay for vaginal fluid was 10 ng/ml, all samples were negative under these conditions)
h: estimated capacity of liquid uptake for the swab: 150 µl, volume used for extraction: 1000 µl (Dale + Custis, 2004)
i: estimated that 10 µl correlate with a 1 cm^2 stain, volume used for extraction: 1000 µl (Dale + Custis, 2004)
j: no reported positive results for female urine with the PSA SEMIQUANT assay have been found in the literature
k: so far one female volunteer has been found whose vaginal swabs showed positive test results with the PSA SEMIQUANT assay dependent on the menstrual cycle (Denison et al., 2004)

Please keep in mind that this table lists the extreme maximum values. The vast majority of the women will exhibit lower concentrations. Generally it can be said, that positive test results with the PSA SEMIQUANT assay are a strong hint that seminal fluid is present, if the case material has been processed in the above described way. This statement is confirmed by the observation that female sample material has been tested consistently negative with the PSA SEMIQUANT assay. Only one exception has been described by Denison et al. (2004) who found positive results with vaginal swabs of one volunteer.

No absolute concentrations of PSA in the vaginal fluid could be found in the literature because extracts from swabs/tampons has been used for the determination. Therefore weak positive results of vaginal swabs should be interpreted carefully. Because the capacity of liquid uptake for the swabs, the volume used for extraction, the extraction buffer and the time used for extraction vary considerably, it is very difficult to compare the PSA values. Therefore vaginal swabs showing weak positive results allow no clear conclusion if seminal fluid is present or not. However, as the vast majority of vaginal swabs showed negative results with the PSA SEMIQUANT assay, even weak positive results should still be considered a strong indicator for sexual assault.

Some of our customers suggested to decrease the sensitivity of the PSA SEMIQUANT Assay to rule out that positive results are caused by other body fluids than semen. For several reasons we do not want to follow these suggestions. If the detection limit is raised from 0.5 to 5.0 ng PSA /ml, this would be of no advantage for the forensic scientist, because he/she would reach the same result by testing a 10fold dilution of the sample. However, the raise of the detection limit would prevent that weak positive results due to degradation or drainage could be detected even if that might be a clear evidence for sexual abuse (e.g. with oral swabs). Furthermore the internal standard allows the forensic scientist to estimate if the sample material contains less or more than 4 ng PSA/ml. Then the interpretation of the result concerning the presence of seminal fluid can be done by the forensic scientist while considering all circumstances of the individual case. We would prefer generating results in this way to blindly increasing the number of negative results by raising the detection limit of the assay.

5) Literature

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6) Annex

Body fluid	PSA-concentration in ng/ml if not indicated differently			source	comment
	mean	median	range		
Seminal fluid			0.2 – 5.5 mg/ml	Hochmeister et al., 1999	Summarized value from several publications
	0.82 ± 0.22 mg /ml		0.07-2.16 mg/ml	Lövgren et al., 1999	
Vaginal fluid		0.11	0.00-1.25	Lawson et al., 1998	Value for extracts of vaginal swabs, no absolute values
	0.43 0.45 0.88		95% Confidential Interval:: 0.26-0.71 0.27-0.74 0.52-1.50	Macaluso et al., 1999	3 independent determinations performed at different times, again values represent extracts of vaginal swabs, no absolute concentrations are given
Saliva			< 0.03 ng/ml[1]	Lövgren et al., 1999	1: Detection limit of the assay, female and male samples
	0.099 ± 0.016[1] 0.048 ± 0.007[2]		0.04-0.34[1] 0.02-0.15[2]	Manello et al., 1996	1: taking oral contraceptives 2: control group
	0,024 ± 0,011[1] 0,029 ± 0,013[2]		< 0.06	Aksoy et al., 2002	Highest values in 1: follicular phase (day 9) or 2: midcycle (day 14)
			129-688	Breul et al., 1993	Single author with such extremely high values, mixed group of patients partly men with urological diseases
Urine (female)	0.29[1]		0.12-1.06[1]	Schmid et al., 2001	PSA detectable in only 11% of the samples, the given values represent these 11 %
	1.73 ± 1.68[1] 12.03 ± 10.47[2]			Breul et al., 1997	1: control group 2: taking of testosterone for gender shift
	7,36			Breul et al., 1993	
	3,72			Breul et al., 1994	
	0.521 ±0.05[1] 0.038 ± 0.005[2]	0.451[1] 0.035[2]	0.09-1.239[1] 0.02-0.15[2]	Manello et al., 1998	1: taking of oral contraceptives, 92 % of samples positive, the given values represent these 92 % 2: control group, 80 % of samples positive, the given values represent these 80 %

	0.0043 ± 0.0018[1] 0.820 ± 0.344[2]		0- 0.046[1] <1 – 10.289[2]	Obiezu et al., 2001	1: control group 2: women with Polycystic Ovary Syndrome (PCOS)
Urine (male)	915.1		21-2,853	Iwakiri er al., 1993	Patients with adenocarcinoma of prostate
			maximally 800	Sato et al., 2002	Estimated value with the PSA SEMIQUANT assay
Serum (female)	0.046 ± 0.07		0.02-0.16	Manello et al., 1996	Taking of oral contraceptives did not change the PSA concentration in the serum
			< 0.6	Filella et al., 1996	58 % of the 276 samples were negative for PSA, only 3 samples > 0.1 ng/ml
	0.032 ± 0.014[1] 0.035 ± 0.015[2]			Aksoy et al., 2002	1: follicular phase 2: midcycle
	0.12 ± 0.02[1] 0.17 ± 0.07[2]			Breul et al., 1997	1: control group 2: taking of high doses of testos-terone for gender shift
	0.004 ± 0.0048[1] 0.043 ± 0.127[2]	0,002[1] 0,004[2]	0 –0.019[1] 0 – 0.579[2]	Megelos et al., 2005	1: control group 2: women with hirsutism
serum (male)			< 4 [1] 4-10 [2] > 10 (partly over 200) [3]	Price et al., 2001	1: healthy men, 2: indication of diseases involving the prostate 3: strong indication for carcinoma of the prostate

PSA was also detected in other body fluids like amniotic fluid, breast milk and breast secretion. For the exact values please see the table on page 14.

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